NEMATODES

- Elongated, cylindrical, unsegmented round worm
- Sexes are separate, diecious
- Head end : No sucker, no hook
- Alimentary canal and anus present, complete
- Body cavity present

General Characteristics

- Body elongate, cylindrical, worm-like and tapered at both ends
- They are unsegmented and pseudocoelom=bodycavity. The pseudocoelom is filled with hemolymph. A fluid filled body cavity acts as a cushion (protection to the organs) and a skeleton.
- Nematodes have seperate sexes (Dioecious); females is usually larger than males. There is a copulatory organ posterior end of the male.
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- Range in lenght from a few mm to several cm or meters long (e.g. 1 mm-120 cm)
- The end of the female is straight and slender.
- Male typically has a coiled tail.

- Bilaterally symmetrical.
- Colorless
- Females > males
- They live different kind of tissue or organs

The body wall is composed of a thick **cuticle**, a **hypodermis** and a layer of longitudinal **muscle** only, there is no circular or diagonal muscle

Cuticle

- The body is covered with a noncellular, highly resistant coating called a **cuticle**. An elastic cuticle covers the body surface of nematodes; it is periodically molted
- Cuticle is secreted by hypodermis
- Specialized structures such as **papillae** (spine-like or finger-like / oral-cervical-caudal), **vesicle, alae** (wing-like / cervical-caudal), **leaf crowns** (external and internal), **plaques** (plate-like), **cordons** (cord-like), **bursal rays** and **copulatory bursa** may be present on the cuticle; these structures may be sensory
- The cuticle not only covers the entire external surface, but it also lines the buccal cavity, esophagus, rectum, cloaca, vagina, and excretory pore

Hypodermis

- Hypodermis secretes the cuticle
- Two lateral cords are the largest and contain the **excretory canal** when these are present, while dorsal and ventral cords contain **longitudinal nerves**

Muscle

- Within and closely associated with the hypodermis are one or more layers of **longitudinally arranged muscle cells (somatic)**
- Locomotion/movement is effected by undulating waves of muscle contraction and relaxation.
- The multiple longitiudinal rows of muscle cells in each of four quarters of a circle is termed **polymyarian**, one with no more than 2 rows of cells is called **holomyarian**, and one with 2 to 5 rows is **meromyarian**
- The cuticle, hypodermis, and somatic musculature make up the **body wall**

Pseudocoelom=Body cavity

- A space within the body cavity allows for the reproductive and digestive systems to evolve more complex shapes and functions
- The pseudocoelom is filled with **hemolymph**. A fluid lined chamber offers protection to the gut and other organs; acts as a cushion
- The fluid filled body cavity acts as a skeleton - hydrostatic skeleton, providing support and rigidity for a soft bodied animal

Digestive System

• Have digestive system. It is complete and tubular
• Consists of an anterior **Mouth** (simple oral opening or large/small buccal capsule may contain teeth, cutting plates), **Oesophagus** (is of variable form, muscular and pumps food into the intestine), **Gut** (is tube whose lumen, contain a single layer of cells and microvilli, which increase the absorbtive capacity of the cells), **Cloaca** (in males there is a cloaca which functions as an anus) or **Anus** (in females the intestine terminates in an anus).

- Ingested from mouth food enters muscular region- esophagus or pharynx
- This is pumping organ that sucks/pumps food into the alimentary canal and into intestine (Because of the hydrostatic pressure of the pseudocoelum which acts on the intestine, the nematodes require a muscular pharynx or oesophagus in order to feed).
- In the muscles of the esophagus have glands- digestive enzymes- amylase, proteases, pectinases, chitinases, anticoagulants.
- Food in parasitic nematodes is blood, tissue cells, body fluid, intestinal contents. Free living nematodes feed on bacteria etc.

Nervous System

- There are 2 major nerve centers in nematodes:
  1. The **circumesophageal nerve ring** or **a central ganglion**
  2. **Nerves** (**dorsal, ventral longitudinal nerves**)

Parasitic nematodes possess both mechanoreceptors and chemoreceptors

1. Mechanoreceptors;

- Located around the mouth are papillae of 2 types: **oral papillae** on the lips surrounding the mouth and **cephalic papillae** behind the lips
- Other papillae may be found at different levels of the nematode body, e.g. **caudal papillae**, observed in many males; aids in copulation

2. Chemoreceptors;

- **Amphids** are chemoreceptors located in shallow anterior pits
- **Phasmids** are a set of chemoreceptors at the posterior end

Excretory System

- The basic component is comprised of 1 or 2 **renettes**, large unicellular glands that empty through **longitudinal excretory canals** and an **excretory pore**
- The renettes and the excretoy pore are usually located anteriorly
- This system has a function in the removal of wastes (It may be strictly osmoregulatory)

Reproductive System

- Reproduce with sexually (copulation)

Male

- One testis. Testis, location of sperm production
- Vas deferens (sperm duct) extends distally to the cloaca
- Vas deferens are evident before it enters the cloaca: the seminal vesicle (sperm storage) and the ejaculatory duct.
- Organelles in copulation: Male nematodes have with one or two copulatory spicules and cuticular structures. The spicules are important during copulation in that they keep the female vulva open, thus facilitating the entry of sperm into the female reproductive tract (Spicules are used to open the vulva for sperm deposition). Other accessory structure may be present, including a gubernaculum and in some species telamon; serves to guide the spicules when they are extended.
- In additon to, caudal alae or copulatory bursa. This structures embrace female during copulation.

Female Reproductive System

- Nematodes generally have 2 cylindrical **ovaries** and **uterus.**

Ovary, location of egg production. Followed by **oviduct** and **uterus** terminating in a vulva. Eggs mature in uterus.

- The uteri unite to form a **vagina** that opens through a **vulva,** usually located near midbody. Vulva, opening to the outside environment.
- Fertilization becomes in **receptaculum seminis**
- The distal portion of the uterus, the **ovojector,** is a short muscular organ, which assists to expel ripe eggs

- **Sexual dimorphism** is present: at the curved posterior end of the male there is a copulatory organ as well as other specialized organs; males are usually smaller than females

- In some species of nematodes females can be **parthenogenic. Parthenogenesis**: The production of offspring from eggs which have **NOT** been fertilized (development of embryos occurs without fertilization by a male).

- Eggs can hatch either within the host or in the external environment. Hatching of eggs in the external environment is , in part, controlled by such environmental factors as temperature, moisture, and oxygen tension
- In some species, the eggs only hatch once they have been ingested by a host. In these cases the stimuli for hatching may be carbon dioxide tension, pH, salts and temperature.

Life style

- Egg
- There are 4 stage larvae/juvenile (L1, L2, L3, L4). Sometimes the sexually immature adult stages are called L5
- Adult
- Egg produced

- Direct life cycle (generally). Nematodes generally live one or two types of lifestyle; free-living or parasitic.
- The third L3 larval stage is normally the infectious stage for parasitic nematodes. 1st and 2nd stage are rhabditiform, 3rd stage is filariform
- Each stages will be ended with molting process. After the final moult, the nematodes will differentiate into males and females. Females can then produce fertile eggs/larvae. Parasitic nematodes live within a host. Free-living nematodes live in environments.

Molting

• Nematodes have 4 molts each of which involves: formation of new cuticle, loosening of the old cuticle, rupturing of the old cuticle, and escape of the larva (The hatched L1 feeds on bacteria and grows until constrained by its outer skin or cuticle. Further growth is possible only if the larva grows a new, more flexible, cuticle and casts off its old outer cuticle. This process is called molting).

• Each stage ends with molting. Cuticle shed between each molting.

• Larval stages in the life cycle of parasitic nematodes are generally referred to as first-, second-, third, and fourth-stage larvae (e.g., L_1, L_2, L_3, L_4).

• This process is controlled by exsheathing fluid secreted by the larva.

Larval Forms

• **Rhabditiform** - The first stage larva of *Strongyloides,* hookworms etc.; the esophagus of this small larva is joined to a terminal esophageal bulb by a narrow isthmus. It is seen in free-living stage (in environment).

• **Filariform** - after molting twice, the rhabditiform larva of *Strongyloides,* hookworms etc. usually retain the remnants of their last cuticle and becom ensheathed, 3rd stage or filariform larva. The esophagus is typically elongate and cylindrical and has no terminal bulb. This larva is usually the stage that is infective to the definitive host

• **Microfilaria** - the prelarvae of filarial worms (e.g. *Dirofilaria immitis, Wuchereria bancrofti, Onchocerca sp.*) are known as microfilariae. The larval body surface is covered by a thin layer of flattened epidermal cells

Classification based on laying eggs or larva

Eggs have characteristic SHAPE, COLOR, SIZE (useful for sp. identification). Larvae identifiable to specific level.

- Oviparous
  - Strongylid type: Eggs with segmented ovum (hookworm, Strongylus and Trichostrongylus species)…a lot of blastomere
  - Ascaridoid type: Eggs with unsegmented ovum (Ascaris species)..one blastomere
  - Oxyuroid type: Eggs contain morula and/or larvae (Enterobius, Oxyuris species). It is flattened on one side
  - Trichuroid type: Eggs with unsegmented ovum with mucus plug at both ends (Trichuris species)

- **Ovoviviparous**
  - **Spiroid type:** Female worms lay **eggs with contain fully formed larvae** (Strongyloides species)

- **Viviparous**
  - **Female worms directly give birth to larvae**; there is no egg stage (Filarial worms, Trichinella species)

Strongylosis

- Most common horse parasites
- Known as redworms or bloodworms
- Adult strongyles live in the large intestine. Divided into large and small strongyles
- Strongylosis is a pasture infection and these worms have direct life cycle.
- Mixed infections are common in horses. In mixed infections, more than 70-80% of strongyle parasites are cyathostomins in Turkey.

- LARGE Strongyles=Migrated: very harmful to horse, 1.5-5 cm
  - *Strongylus equinus*
  - *Stronglus edentatus*
  - *Strongylus vulgaris* ("verminous arteritis" "thromboembolic colic")

- SMALL Strongyles (cyathastomes)=Non-migrated: 1-2.5 cm
  - More than 40 species are in this group

Buccal capsule in large strongyles

Strongylus equinus; a large dorsal and 2 smaller sub-ventral conical–shaped teeth
S. vulgaris; two dorsal ear-shaped teeth
S. edentatus; no teeth

Life cycle in free-living stage both small and large strongyles (on pasture)

- Generally, mixed infection is common in Turkey and in the world. There are both free-living and parasitic stage.
- *Strongyles* worms (large and small) have a **direct life cycle.**
- Adult worms lay eggs in the large intestine. Eggs are shed with the feces.
- L1 develops in the eggs and release from egg.
- Larvae develop to infective 3th stage within 1-2 weeks in the pasture.
- Infective larvae (L3) are ingested with grass or water by the horse
- Once ingested, larvae travel through the body. Life cycle and behavior is species-specific.
- Prepatent period (time between infection and first eggs shed) is 6-12 months in large strongyles. This period is 2-4 months in small strongyles.

Life cycle and behavior is species-specific (Large Strongyles) in parasitic stage (in the final host)

- Infection occurs by ingestion of infected grass. The 3th stage larvae enter the mucosa of intestine
- Migrating larvae of Strongylus sp. can be found in several organs: particularly in blood vessels, lungs, liver, pancreas, etc. depending on the species and the developmental stage.

- *S. equinus*……migrate to the liver and pancreas, and back into the LI

- *S.edentatus* …migrate to the liver and retroperitoneal organs, and back into the LI

- *S.vulgaris* ……migrate to the cranial mesenteric arteries (within the vascular system), and back into the LI

Clinical signs and pathogenesis in large strongles

- Adult worms can cause diarrhea, dehydration, colic, fever, oedema, anemia, loss of appetite, depression and weight loss. In severe cases gangrenous enteritis, rupture and intestinal infarct may happen, with a fatal outcome.
- *S.equinus & S.edentatus* larvae can't generally cause clinical signs but, *S. vulgaris* larvae lead to tromboarteritis and aneurysm in A.m.c. and caecal-colic arteries. Thrombose can cause emboli, infarction and necrosis of intestine, and lead to severe colic. Wall damage causes hemorrhage, that favors clotting. Some clots break off and are transported elsewhere they may obstruct the blood flow (thrombosis).
- If these affects the hindlimbs (A.femoralis) increasing weakness up to lameness (one or both sides) may occur upon exercise. Symptoms often recede after resting. In severe cases atrophy of the hind limb muscle may occur, as well as plugging of the distal aorta, with acute paralysis and recumbency. Aneurysms can suddenly cause with fatal outcome within minutes if they affect important blood vessels.

In contrast with the large strongyles, larvae of the **small strongyles=cyathostomes do not migrate out of the intestine**.

- Small strongyles are less harmful than large strongyles
- After ingestion by a grazing horse, larvae travel through the large intestine.
- L_3 cyathostomins enter the mucosa of the LI. Within 1 to 2 weeks after infection, a fibrous capsule develops around the larva, which is called as "encysted" .
- The third-stage larvae (L_3) grow, then molt into (L_4) fourth-stage larvae (It takes usually 30-60 days).
- The L_4 stage emerges from the cyst into the lumen of the large intestine and develops into adult nematode. Prepatent period is 2-4 months.
- In some cases (drought and cold), 3th stage larvae enter hypobiosis and emerge later as L4 (It takes several months or even years).
- Larval development of small strongyles may be produce an adult worm in as quickly as 5 to 6 weeks, or larvae may arrest development in the L_3 stage, and not undergo further maturation for up to 2.5 years.

Clinical signs in cyathostomes= small strongyles

- Clinical signs is associated with simultaneus mass emergence of cyathostomins L4 from mucosa = "larval cyathostominosis" . This is a seasonal condition. Subclinical diseases is more common and may result in greater economic losses.
- Syndromes are severe diarrhea unresponsive to treatment, rapid weight loss, loss of condition, recurrent colic , hypoproteinemia, and view of numerous larval cyathostomins in the feces.
- Larval cyathostominosis occurs frequently in late winter or early spring, when encysted larvae are expected to emerge from arrested development.

Larvae enter mucosa of intestine and form nodules, they do not migrate any further

- A large proportion of encysted larvae emerge from gut tissues during late winter or early spring and mature into adult cyathostomins.
- The fecal egg counts increase in spring.
- Parasites continues to reproduce through the summer months, when conditions are favorable for egg hatching and larval development.
- The majority of larvae ingested during autumn are pass on to arrested development within the host.
- This parasites overwinters within host tissues and emerges in the following spring to initiate another annual cycle.

Diagnosis

- Fecal floatation
- Fecal Egg Count
- Fecal culture
- Necropsy

- ✓ Infection with adult strongyles is easily demonstrated by using faecal flotation techniques to detect strongylid eggs in the feces.
- ✓ Large strongyle eggs cannot be differentiated from cyathostomes eggs. So, egg cannot be differentiated in fecal analyses
- ✓ Difficult to detect cyathostomes infection in feces. Epg is not reliable in fecal diagnosis (low or not). Clinical signs, season, age of the horse are important.
- ✓ Medium-sized egg (70-140 X 40-65 µm), oval, thin-shell, contain 8-16 cells with large blastomere (morula)
- ✓ Identification of species is done through fecal culture (allows identification of larvae).

Diagnosis

- 20% of the horses shed 80% of the worms (young horses)
- Fecal exam only detects eggs from adult females
- Eggs per gram of feces (with McMaster Technique)
- Utilize Fecal egg counts to determine low and high shedders ….200 or less EPG is a light load – may not need to treatment
- A parasite-free horse is an anomaly and not a realistic goal
- Provides vital information about the individual as well as the parasite status of the herd

Drug Resistance

- Each time a horse is treated, the resistant worms live to shed their eggs onto the pasture
- Resistance to dewormers is a problem
- Resistance seen to all 3 classes of dewormer drugs
    - Large Strongyles – adult stages –can use all class of dewormers, larvae (moxidectin, ivermectin or fenbendazole)
    - Small strongles – very susceptible to resistance- 98 percent resistant to some class of drugs
- Decrease of refugia of parasites can lead to resistance development (Refugia= proportion of parasite population not affected by treatment)

			Anthelminthic Efficacy			
Compound	**Dosage**	**Safety index**	**Large Strongyles Adults**	**Small Strongyles Adults**	**Migrating larvae**	**Encysted larvae**
Moxidectin	0.4 mg/kg p.o.	>5	+	+	+	+
İvermectin	0.2 mg/kg p.o.	10	+	+	+	-
Dichlorvos* **	35 mg/kg p.o.	3	+	+	+	
Trichlorfon* **	40 mg/kg p.o.	1				
Pyrantel pamoate	6.6 mg/kg p.o.	20	+	+	+	
Pyrantel tartrate	12.5 mg/kg p.o.	>100	+	+	+	
Thiabendazole*	44 mg/kg p.o.	13	+	±	+ 10X, 2 days	
Mebendazole*	8.8 mg/kg p.o.	45	+	-		
Fenbendazole*	5 mg/kg p.o.	200	+	±	+ 2X, 5 days	+ 2X, 5 days
Cambendazole**	20 mg/kg p.o.		+	±		
Oxfendazole*	10 mg/kg p.o.	10	+	±	+ 2X, 1 days	
Oxibendazole*	10 mg/kg p.o.	60	+	±		
Febantel	6 mg/kg p.o.	33	+	±		
Piperazine	88 mg/kg p.o.	17	-	±		

+: 90-100% efficiacy,…… ±: % 90-100 If the drug resistance has not occured,…….. −: % 10-80

***: don't use in the first 3 months of pregnancy,……. **: don't use in pregnant,…… ***: don't use in foals and pregnant**

Determining Resistance

- Fecal egg count reduction test (**FECRT**)-compares the number of parasite eggs in the feces after treatment with the number that were there before treatment

$$\frac{\text{EPG (Pre-treatment)- EPG (10-14 day post-teatment)}}{\text{EPG (Pre-treatment)}} \times 100 = \text{FECRT}$$

Should be >90% - if not (< 80%), there is resistant or if not (80-90%), it is suspicious.

- Egg reappearance period (**ERP**)- is the time interval between the last effective treatment and the resumption of parasite eggs in the feces

- Ivermectin……..9-13 w (ERP when drug was first used)…….6-8 w (Usual ERP when drug is effective)
- Moxidectin…….16-22 w («)…….10-12 w («)
- Pyrantel……5-6 w («)…….4-5 w («)
- Benzimidazol….6 w («)……..4-5 w («)

If the duration of ERP is shortened, resistance is developing.

Ways to Avoid Resistance

- Regular fecal examinations - fecal float
- Treat with proper anthelmintic - keep FEC low (only for strongyles and roundworm)
- Give the correct dose of dewormers
- Rotate between dewormers of different drug classes
- Reduce the development of drug resistance…horses that have low egg shedding (0-200 epg) should not treated (with fecal egg counts)
- Proper timing of treatment (infective larvae at their lowest numbers in the winter, deworming during these unnecessary periods increases resistance)
- Cleanliness……reduce transmission (Transmission occurs almost exclusively on pasture.)
  - Manure management (pile and compost manure)
  - Pick up dung/feces at least twice a week
- Try and determine which horses are more at risk or have weaker immune systems
- Pasture management=Adecuate pasture area (avoid overgrazing pasture), pasture rotation (mixed grazing-cattle and horse, group horse by age), clean pastures for young (foals, young horses should go to the cleanest available pasture)
- Keeping treated horses off pasture for 2-3 days post treatment

Oesophagostomum-in Ruminant

- Adult worms live in the large intestine.
- These worms are known «nodular worm disease»
- Mainly lambs and calves
- Size of worm is 1-2.5 cm
- Cervical alea with «leaf crown»
- Oesophagostomum has direct life cycle and a pasture infection
- Prepatent period 4-6 weeks

Sheep & Goat	*O.columbianum, O. venulosum,*
Cattle	*O. radiatum*
pig	*O. dentatum,* *O.quadrispinulatum*

Life cycle and clinical signs

- Preparasitic stage is typically strongylosis.
- After ingestion by grazing ruminant, 3th stage larvae travel through the intestine. Larvae only migrate in the intestine of the host.
- L_3 enter the mucosa of the SI and, then molts into L_4 (1st histotropic phase) and then return to the lumen. Then some of L_4 enter the LI mucosa (2nd histotropic phase) or some of L_4 develop into the lumen.
- ✓ On reinfection with some species (O.c.,O.r.) larvae may remain arrested as L4 in nodules for 3-4 moths or up to 1 year. According to the bad immunity (birth, lactation, bad care, malnutrition, other infections..), the L_4 emerges from the cyst and develops into L_5 and adult nematode.
- The main damage is attributed to the nodule forms in the intestine. They can cause;
  - Extremely bad smelling and severe persistent diarrhea
  - Loss of appetite, weight loss, anemia
  - Death in young animals

Pathogenesis

- The most serious problems seen in Oesophagostomum infections arise from larvae penetrating the musosa of the intestine. If the immunity in sheep is well developed, the number of nodules is high but, number of adult are low. Larvae emerge from nodules in bad care and malnutrition of animals. The larvae in the nodule die over time.

- After initial infections, small nodules about 1 mm in diameter form around larvae in the mucosa. When larvae move back into the intestinal lumen the remaining nodules may be hemorrhagic particularly in acute infections but often they fill with purulence, in which cases they are more properly described as small abscesses.
- In heavy infections, the mucosa becomes inflamed and edematous and regional lymph nodes are often much enlarged.
- Chronic infections will produce an intestinal mucosa that is filled with nodules particularly if these repeat infections have been heavy. In these chronic cases tissue reactions are more severe and the nodules are much larger (2-3 cm/sheep, up to 5 cm/cattle in diameter) and creamy in color due to the development of connective tissue around them. In chronic infections, most infecting larvae will be killed by host reactions. Therefore older animals will usually show extensively nodular intestines but with few, if any, adult worms in the colon.

- **Acute disease**: **Associated with L3-L4 molt and L4 emergence 8-10 after infection→ Fetid diarrhea, slough tags of mucosal tissue, can die within 1-3 weeks**

- ***Chronic disease:*** **Several nodules per square inch, < gut motility; 3000 adults have major effects; Nodules increase in size, number with immunity. Adults+late L4 → anemia, diarrhea, debility, severe anorhexia (>50%) Inflammation with edema → Protein, fluid, RBC loss. An 'ulcerative colitis > 3 weeks after heavy infection**

- The most pathogen species are *O. columbianum.* In older animals or on reinfection, larvae may remain arrested in the nodules as hypobiotic L_4 for up to 1 year.
- These infection cause economic loses.

Diagnosis and treatment

- Infection with Oesophagostomum is demonstrated by using faecal flotation techniques to detect eggs in the feces.
- The egg is typical «trichostrongylid type». But, egg cannot be differentiated from other trichostrongylid eggs.
- Medium-sized egg (70-120X40-60 μm), oval, thin shell, egg contain 8-32 cell blastomeres
- Identification of species is done through fecal culture.

- Benzimidazole, ivermectin, doramectin, moxidectin, abamectin, levamisole, fenbendazole…